Dietary fat and menstrual-cycle effects on the erythrocyte ghost insulin receptor in premenopausal women[1-3]

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ABSTRACT The effect of high- and low-fat diets with different levels of fatty acid unsaturation on insulin receptors of erythrocyte ghosts was studied during different phases of the menstrual cycle in 31 healthy premenopausal women. Subjects were divided into two groups and consumed controlled diets containing 39% fat with a ratio of polyunsaturated to saturated fatty acids (P:S) of either 0.30 or 1.00 for four menstrual cycles. They were switched to 19% fat at the same P:S for another four cycles. Fasting blood samples were collected during the follicular and luteal phases. Insulin receptors were measured from right-side-out ghosts. Insulin binding was significantly lower due to fewer receptors when subjects were fed the low-fat, high-carbohydrate diet compared with the high-fat, low-carbohydrate diet. There was no significant effect of level of unsaturation or time of menstrual cycle on insulin binding. Thus, insulin receptors on erythrocytes respond to dietary lipids. *Am J Clin Nutr* 1989;50:460–4.

KEY WORDS Insulin receptor, high-fat diet, low-fat diet, erythrocyte ghosts, menstrual cycle

Introduction

The type and amount of dietary lipids (1–4) and dietary carbohydrates (5–7) are known to affect plasma lipid levels and lipid metabolism in humans and animals. Dietary carbohydrates and lipids also alter plasma levels of the hormones involved in their metabolism. Thus, a low-fat, high-carbohydrate diet stimulates insulin secretion more than a high-fat, low-carbohydrate diet whereas the opposite is true for glucagon release (8) in healthy volunteers. Because hormones act via their receptors it is of interest to study whether insulin receptors are also altered by the type and amount of dietary lipids.

Insulin receptors are affected by the phase of the menstrual cycle (9). It is not clear, however, whether the effects of the type and amount of dietary lipids on insulin receptors would be the same throughout the menstrual cycle. This report examines the effect of type and quantity of dietary fat on insulin receptors in premenopausal women during the follicular and luteal phases of the menstrual cycle.

Methods

Thirty-one premenopausal women aged 20–40 y were studied over a 9-mo period. Initial baseline data were collected during a period of one menstrual cycle when the subjects were consuming self-selected (SS) diets. Composition of the SS diets is given elsewhere (4) and was very similar to the high-fat, low-carbohydrate (HF-LC) diet. Subjects, paired according to their age and relative body weight, were randomly assigned to one of two dietary groups based on a ratio of polyunsaturated to saturated fatty acids (P:S) of either 0.3 (15 subjects) or 1.0 (16 subjects). The subjects maintained the assigned P:S throughout the study. Both groups were placed on an HF-LC diet (39% of energy from fat and 45% from carbohydrate) for four menstrual cycles, followed by a low-fat, high-carbohydrate (LF-HC) diet (19% of energy from fat and 64% from carbohydrate) for an additional four menstrual cycles.

During the controlled dietary periods all meals were prepared in the Human Study Facility of the Beltsville Human Nutrition Research Center (BHNRC). On weekdays breakfast and dinner were served in the BHNRC dining facility and carry-out meals were provided for weekday lunches and all weekend meals. A 14-d menu cycle formulated from commonly

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available foods (4) was used. Menus were designed for four caloric intake levels: 1600, 2000, 2400, and 2800 kcal. Subjects consumed no vitamin-mineral supplements or alcohol during the study. The experimental protocol was approved by the Institutional Review Boards of the Georgetown University School of Medicine, the US Department of Agriculture, and the National Cancer Institute.

For the determination of erythrocyte-ghost insulin receptors, morning fasting venous blood samples were collected in evacuated tubes containing EDTA (1.4 mg/mL; Sigma Chemical Co, St Louis, MO) and Trasylol® (100 U/mL; FBA Pharmaceuticals, New York) during the midfollicular (proliferative) phase and also during the midluteal (secretory) phase, as determined by the length of the menstrual cycle. Samples were collected during the fourth cycle in each of the two controlled diet periods in addition to the prestudy period. Right-side-out ghosts were prepared from erythrocytes [by lysing erythrocytes in a hypotonic phosphate buffer as described by Dodge et al (10)] and were then stored at −70 °C until studied. Insulin binding was measured by incubating ghosts (100 μg protein/tube) in 0.5 mL Tris-HEPES buffer (11) containing 40 μg bacitracin/mL (gift from Upjohn Co, Kalamazoo, MI) at 4 °C for 16–18 h with 0.1 ng ^{125}I-labeled insulin (specific activity 81.4 TBq/mmol) and 0–100 μg native porcine insulin/mL. ^{125}I-labeled insulin was purchased from New England Nuclear (Boston, MA) and native porcine insulin was a gift from Eli Lilly & Co (Indianapolis, IN). After the incubation, 0.2-mL aliquots were layered over 0.2 mL chilled Tris-HEPES buffer and centrifuged for 60 s at 7500 × g (Microfuge II, Beckman Instruments, Inc, Fullerton, CA). The ghost pellet was washed once with 10% sucrose and radioactivity was determined in a gamma counter (model A5550, Packard Instrument, Downers Grove, IL). Binding data were analyzed by Scatchard plots (12) and competition-inhibition plots (13). The number of receptors was assessed from the intercept on the abscissa of the Scatchard plots and the affinity was determined from the competition-inhibition plots as the amount of native insulin required to displace 50% of bound tracer.

The design of the study was a split-split plot. A split-split plot design recognizes three levels of variation (among subjects, within subjects among periods, and within subjects among periods between menses). The data were analyzed with a general linear models procedure (14). Because replicates were unequal, least-square means were calculated. Least-square means are means that have been corrected for unequal replication in the study to make fair comparisons between all the groups. Data from the SS dietary period were analyzed separately from the controlled periods because no P:S treatment was applied during the controlled periods. Comparisons of SS, LF-HC, and HF-LC diets were made using the least-significant-differences technique (14).

Results

Insulin binding to erythrocyte ghosts was significantly affected by the amount of dietary fat and carbohydrate; feeding the LF-HC diet decreased specific insulin binding compared with the HF-LC diet. There were no significant differences when subjects were switched from their preselected diet to the HF-LC diet (**Table 1**). No significant differences were observed between groups fed the relatively more saturated (P:S of 0.3) or more unsaturated (P:S of 1.0) dietary fats. Furthermore, there were no significant differences in insulin binding between follicular and luteal phases.

Scatchard analysis of the data showed that the decreased insulin binding on the low-fat, high-carbohydrate diet was due to a significant decrease in the number of receptors (**Table 2**). The number of receptors was not affected by either the degree of unsaturation of the dietary fat or the phase of the menstrual cycle during which the sample was collected. When the binding data were analyzed by competition-inhibition plots, the graph was shifted to the left when the subjects were consuming the low-fat, high-carbohydrate diets compared with when they were consuming either the self-selected or the high-fat, low-carbohydrate diets. This means that less native insulin was required to displace bound hormone when subjects consumed the low-fat, high-carbohydrate diet as compared with the other two diets, indicating a higher affinity for insulin. The quantitative data are presented in **Table 3**. The affinity of insulin receptors was not influenced by either the level of unsaturation of the dietary fat or by the phase of the menstrual cycle.

Plasma insulin levels were significantly higher when the subjects were consuming the LF-HC diet (84.6 ± 2.6 pmol/L) than when they were consuming either SS diets (74.6 ± 2.6 pmol/L) or the HF-LC diet (76.6 ± 2.5 pmol/L), as reported previously (15). Plasma insulin levels were also significantly higher during the luteal phase (84.5 ± 2.2 pmol/L) than during the follicular phase (72.7 ± 2.0 pmol/L) (15).

Discussion

The effect of dietary fats on the binding of insulin to adipocytes, monocytes, and erythrocytes has been studied by us and others (16–23). This study, however, is the first to report the effect of the amount and nature of dietary fat in isocaloric diets on insulin binding to erythrocyte ghosts in premenopausal women. The major observation of this study was the decrease in insulin binding when the amount of dietary fat was lowered and dietary carbohydrate was increased. The reduced binding was due to changes in both the number and affinity of the receptors. A negative correlation ($p < 0.05$) was observed between plasma insulin levels and insulin binding to erythrocyte ghosts in women fed different levels of dietary fat and carbohydrate. Thus, feeding the low-fat, high-carbohydrate diet increased plasma insulin and decreased insulin binding to erythrocytes ghosts.

Our data differ somewhat from other research on insulin binding to other cells. Beck-Nielsen et al (16) reported a significant decrease in insulin binding to monocytes when young healthy volunteers were fed excess calories from fat for 1–2 wk. No change in plasma insulin was observed. A similar decrease in insulin binding was observed when subjects were fed excess calories from sucrose, which increased plasma insulin levels. Feeding isocaloric diets with low fat had no significant effect on insulin binding or plasma insulin levels whereas feeding a

TABLE 1
Effect of level and unsaturation of dietary lipids on insulin binding to erythrocyte ghosts in premenopausal women*

P:S†	Menstrual-cycle phase‡	Dietary period§		
		Self-selected (SS)	High-fat, low-carbohydrate (HF-LC)	Low-fat, high-carbohydrate (LF-HC)
0.3	Follicular	1.99 ± 0.16	2.01 ± 0.15	1.65 ± 0.16
0.3	Luteal	2.20 ± 0.14	1.86 ± 0.16	1.43 ± 0.17
1.0	Follicular	2.02 ± 0.15	2.18 ± 0.14	1.63 ± 0.17
1.0	Luteal	2.51 ± 0.15	2.05 ± 0.14	1.87 ± 0.17

* Least-square means ± standard error of least-square means of percent specific binding per 100 μg protein.
† Ratio of polyunsaturated to saturated fatty acids in the experimental diets.
‡ Blood samples were collected during the midfollicular phase and during the midluteal phase.
§ Because significant effects were seen only for period, only period means were compared. The mean values during the SS (2.18 ± 0.07) and HF-LC (2.03 ± 0.07) diets were significantly different from the mean value during the LF-HC diet (1.65 ± 0.08) according to least-square means ($p < 0.05$).

low-sucrose diet increased insulin binding. It thus appears that decreased binding to monocytes was a function of excess calories and was not due to either the high-fat or high-carbohydrate diet. Garrel et al (17) reported a small decrease in insulin binding to erythrocytes of healthy human volunteers when they were fed 15% fewer calories. In rats, feeding a high-fat diet for 5–10 d decreased insulin binding to soleus muscle (18) and adipocytes (19). No consistent change was observed in plasma insulin; a high-fat diet increased plasma insulin in the former study (18) and decreased it in the latter (19).

In our study both the LF-HC and HF-LC diets contained similar ratios of simple to complex carbohydrates. Hjollund et al (20) reported increased insulin binding to adipocytes and monocytes in noninsulin-dependent diabetics without a significant change in plasma insulin levels when they were fed a low-fat, high-starch, high-fiber diet. However, no change in insulin binding to erythrocytes was observed. It thus appears that changes in insulin binding to erythrocytes are not as uniform in response to diet as is binding to monocytes, adipocytes, or soleus muscle; adipocytes and soleus muscle are classical target tissues for insulin.

Feeding diets high in saturated fats lowers insulin binding to rat adipocytes (19, 21), minipig erythrocytes (22), and rabbit erythrocyte ghosts (23). A similar effect of saturated fatty acids on insulin binding was observed in cultured cells (24–26) and in reconstituted unilamellar liposomes (27, 28). In the present study insulin binding to erythrocyte ghosts was lower in women fed a diet with more saturated fat (P:S of 0.3) than those fed more unsaturated fat (P:S of 1.0); however, the differences were not statistically significant. Plasma insulin levels were also slightly lower in women fed more saturated fat (15).

The menstrual cycle affects insulin binding to monocytes (9) but has no effect on plasma glucose or insulin (29). In the present study we observed significantly higher insulin binding during the follicular phase compared with the luteal phase in the prestudy period only ($p < 0.03$). This difference disappeared when the women

TABLE 2
Effect of level and unsaturation of dietary lipids on the number of insulin receptors on erythrocyte ghosts of premenopausal women*

P:S	Menstrual-cycle phase	Dietary period†		
		Self-selected (SS)	High-fat, low-carbohydrate (HF-LC)	Low-fat, high-carbohydrate (LF-HC)
0.3	Follicular	11.3 ± 0.9	10.8 ± 0.9	7.4 ± 0.9
0.3	Luteal	11.9 ± 0.9	14.8 ± 0.9	6.4 ± 1.0
1.0	Follicular	9.1 ± 0.9	11.5 ± 0.9	6.9 ± 0.9
1.0	Luteal	11.4 ± 0.8	11.9 ± 0.9	7.7 ± 0.9

* Least-square means ± standard error of least-square means of number of receptors (fmol of insulin bound/100 μg protein. Values are derived from individual plots at each time period for each subject.)
† The mean values during the SS (11.0 ± 0.3) and HF-LC (12.2 ± 0.3) diets were significantly different from the mean value during the LF-HC diet (7.2 ± 0.5) according to least-square means ($p < 0.05$).

TABLE 3
Effect of level and unsaturation of dietary lipids on the affinity of insulin receptors on erythrocyte ghosts of premenopausal women*

P:S	Menstrual-cycle phase	Dietary period†		
		Self-selected (SS)	High-fat, low-carbohydrate (HF-LC)	Low-fat, high-carbohydrate (LF-HC)
0.3	Follicular	160 ± 19	124 ± 15	64 ± 15
0.3	Luteal	136 ± 17	152 ± 15	96 ± 17
1.0	Follicular	141 ± 19	134 ± 14	88 ± 14
1.0	Luteal	153 ± 17	121 ± 12	84 ± 15

* Least-square means ± standard error of least-square means of affinity of the receptors (measured from the competition-inhibition plots as the amount of native insulin [pmol/L] required to displace 50% of the bound tracer). Values for each subject are derived from individual plots. A higher number indicates a rightward shift in the competition-inhibition plot, thereby indicating a decrease in the affinity of the receptors.

† The mean values during the SS (172 ± 9) and HF-LC (132 ± 7) diets were significantly different from the mean value during the LF-HC diet (83 ± 7) according to least-square means ($p < 0.05$).

were fed the controlled diets. Plasma insulin levels were accordingly lower during the follicular phase (15).

The data from this and several other studies (13, 16, 19, 20) indicate that insulin binding to erythrocyte ghosts, adipocytes, muscle, liver, and monocytes has variable results. In many studies both similar and different insulin-binding data have been observed between different tissues as discussed above, even between monocytes and erythrocytes in humans (13). In most instances in the present study, insulin binding showed an inverse relationship with plasma insulin whereas in other studies this was not the case (16, 19, 20). It is possible that erythrocytes can be altered during the preparation of ghosts because, depending on the presence or absence of divalent ions (especially Ca^{++} and Mg^{++}), either right-side-out or inside-out ghosts result. It is important to note that though insulin binding can be measured from right-side-out ghosts (as prepared in this study), it is difficult to assess kinase activity in such preparations because the activity is present on the inner surface of the membranes.

Insulin binding to membranes is dependent on the membrane lipid environment, which determines the fluidity. In a study of the same human subjects reported elsewhere (30) we observed a significant direct correlation between membrane fluidity determined by fluorescence polarization and insulin binding ($p < 0.001$), which confirms our previous observations in minipigs and rabbits (22, 23). Thus, insulin binding to erythrocytes is altered by dietary lipids and also reflects fluidity of the membranes.

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